Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/AU05/000280

International filing date: 28 February 2005 (28.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/548,118
Filing date: 27 February 2004 (27.02.2004)

Date of receipt at the International Bureau: 19 April 2005 (19.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

AU/05/280

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APPLICATION NUMBER: *60/548,118*
FILING DATE: *February 27, 2004*

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PATENT APPLICATION SERIAL NO. _____

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FEE RECORD SHEET

03/03/2004 MBIZUNES 00000043 60548118
01 FC:1005 160.00 OP

PTO-1556
(5/87)

*U.S. Government Printing Office: 2002 — 489-267/69033

Mail Stop Provisional Patent Application

15750 U.S.PTO

PTO/SB/16 (6-95)
Approved for use through 04/11/98. OMB 0651-0037
Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION COVER SHEET

10/227042uest for filing a PROVISIONAL APPLICATION under 37 CFR 1.53 (c).

	Docket Number	47-205	Type a plus sign (+) inside this box→	+

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22387 U.S.PTO 60/548118 022704

TITLE OF THE INVENTION (280 characters)

TARGET FOR LYMPHOPROLIFERATIVE DISORDERS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification — Number of Pages: 39
☒ Drawing(s) — Number of Sheets: 3
☐ Applicant claims "small entity" status.
☐ "Small entity" statement attached.
☐ Other (specify)

METHOD OF PAYMENT *(check one)*

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☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE _____ 25,327 DATE **February 27, 2004**

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Target for lymphoproliferative disorders

Field of the Invention

The present invention relates to the diagnosis and treatment of lymphoproliferative disorders such as multiple myeloma (MM). In particular, the present invention relates to the treatment of lymphoproliferative disorders using ligands which bind to the lambda myeloma antigen (LMA).

Background of the invention

Multiple myeloma is a cancer of the blood in which the malignant cell is a terminally differentiated monoclonal B cell. Conventional treatment of this disease is a high dose chemotherapy regime with or without autologous stem cell transplantation. However, there is now overwhelming clinical evidence that this treatment regime will inevitably fail because the tumour will ultimately become refractory (Davies *et al.* 2000, Ryoo *et al.* 2002, Kyle, 2001a).

Current Treatments for Multiple Myeloma

Current therapies for MM have been reported in the literature and include variations of high-dose chemotherapy (Kyle RA, (2001a) The Oncologist 6; 2; 119-124; Kyle RA, (2001b) Seminars in Hematology 38; 2; 3; 11-14, Anderson *et al.* (1999) Seminars in Hematology 36; 1; 3-8). Most patients with MM have symptomatic disease at diagnosis and require therapy, however, some patients are asymptomatic and are generally not treated until they become symptomatic.

The treatment of choice for MM patients younger than 65 years is autologous peripheral blood stem cell transplantation (APBST) in combination with chemotherapy (Harousseau and Attal (2002) Blood Reviews 16; 245-253). Chemotherapy alone is the preferred initial treatment for patients older than 65 years or younger patients for whom transplantation is not feasible. APBST is applicable for more than half of patients with MM. Despite attempts to reduce tumour cell contamination of the grafts it has been shown that autologous peripheral stem cells are generally contaminated by myeloma cells or their precursors. This results in re-population of the bone marrow with malignant cells and ultimately in relapse.

Initial treatment for symptomatic MM patients is high-dose chemotherapy (Kyle RA, (2001a) The Oncologist 6; 2; 119-124; Kyle RA, (2001b) Seminars in Hematology 38; 2; 3; 11-14, Anderson *et al.* (1999) Seminars in Hematology 36; 1; 3-8). Most physicians treat the patients with vincristine, doxorubicin (Adriamycin) and dexamethasone (VAD) for 3-4 months. This results in a reduction of tumour cells in the bone marrow and peripheral blood. High dose cyclophosphamide and granulocyte-colony stimulating factor (G-CSF) are then administered. G-CSF stimulates the production of peripheral stem cells (CD34+ B-cells) for autologous peripheral blood stem cell transplantation. At this stage peripheral blood is taken and stem cells are collected using a fluorescence activated cell sorter (FACS).

Currently there is a choice of at least two treatment regimes prior to bone marrow engraftment with stem cells. In the first case the patient can be given high-dose chemotherapy and/or total body irradiation followed by APBST. Alternatively the patient can be given alkylating agents after stem cell collection until a plateau is reached. Then α_2-interferon can be given to inhibit cell growth and division, or no therapy can be given until relapse. At this stage the patient is given high-dose melphalan and/or total body irradiation and the previously collected blood stem cells infused. In general, melphalan (200 mg/m^2) is given as it is less toxic and there appears to be no advantage in using total body irradiation.

Comparative studies of conventional chemotherapy and high dose chemotherapy in combination with APBST in MM patients indicated that the latter significantly improved the event free survival and overall survival (Harousseau and Attal (2002) Blood Reviews 16; 245-253). At present VAD treatment followed by APBST results in a favourable five-year survival rate in the transplantation group versus VAD treatment alone (52% vs 12%). Chemotherapy can be continued until the patient is in a plateau state or for one year. If relapse occurs in the plateau stage after 6 months the chemotherapy regime should be re-instituted. Despite the recent improvements in treatment regimes, long-term follow up of these clinical studies has shown that elimination of myeloma from the patient does not occur even with large doses of chemotherapy and APBST.

Thus the current treatment extends the life of the patient but is not curative. It is now apparent that disease progression is associated with genetic instability and more

specifically with dysregulation of genes involved in adhesion, apoptosis, cell cycle, drug resistance, growth arrest, oncogenesis, signaling and transcription (Zhan *et al.* (2002) Blood 99; 5; 1745-1757).

Disease Progression is a Multistep Transformation Process

Several studies have suggested that disease progression in multiple myeloma correlates with progressive genetic events in the malignant plasma cell (Hallek *et al.* (1998) Blood 91, 1; 3-21; Avet-Loiseau *et al.* (2002) Blood 99; 6; 2185-2191; Zhan *et al.* (2002) Blood 99; 5; 1745-1757). The progressive stages of the disease appear to be initiated by a pre-existing monoclonal plasma cell disorder referred to as monoclonal gammopathy of undetermined significance (MGUS) where the cells are immortalized, but not transformed. The next stage of progression is intramedullary myeloma where the cells are found only in the bone marrow and are dependent on bone marrow stromal cells (BMSCs) for survival. In particular, a paracrine loop for interleukin-6 (IL-6) and the interleukin-6 receptor (IL-6R) develops between MM cells and the BMSCs. IL-6 appears to be the most important cytokine in establishing myeloma cells in the bone marrow. The most marked effect is the ability of IL-6 to inhibit dexamethasone-induced apoptosis in myeloma cells.

Concomitantly, myeloma cells stimulate osteoclasts that are responsible for bone resorption resulting in the characteristic bone lesions found in MM. Following this stage is an extra-medullary phase where the cells proliferate more rapidly and grow in the blood (plasma cell leukaemia, PCL) or other extra-medullary sites. The final stage of development is where cells become completely dysregulated and may be propagated *in vitro*.

Genetic Abnormalities

Disease progression at the cellular level is closely linked to genetic abnormalities that are associated with specific examples of gene dysregulation (Hallek *et al.* (1998) Blood 91, 1; 3-21). Several karyotypic studies have shown that aneuploidy is a common characteristic of myeloma cells and is independent of disease stage. A review of these studies has suggested that there are two major categories of genetic abnormalities in multiple myeloma (Fonseca *et al.* (2004) Cancer Research 64; 1546-1558). One category consists of patients with translocations involving the immunoglobulin heavy

chain locus (IgH) which accounts for approximately half the genetic abnormalities in myeloma patients. It is also clear that the hypodiploid karyotypes and chromosome13 monosomy are commonly associated with IgH translocations. The prevalence and clinical importance of specific IgH translocations have recently been determined and are reported in Fonseca *et al.* (2004) Cancer Research 64; 1546-1558.

The remaining 50% of patients appear to have the hyperdiploid karyotype and do not have IgH translocations.

Translocations that involve the light chain (*IgL*) genes have not been well characterised. One study has indicated that IgL-λ translocations are present in approximately 10% of MGUS samples and approximately 20% of intramedullary MM tumours (Fonseca *et al.* (2002) Blood, 100; 1417-1424). Translocations of IgL-κ have only been identified in a small number of tumours from intramedullary MM (Fonseca *et al.* (2004) Cancer Research 64; 1546-1558). At present, the clinical importance of IgL translocations is unknown.

The monoclonal protein of myeloma cells

Despite the complexity of the karyotypic abnormalities found in multiple myeloma, a laboratory hallmark of this disease is the production and secretion of monoclonal protein (M-protein) into the blood and/or urine. In general the M-protein is an immunoglobulin, or a component of an immunoglobulin, that has not retained normal antibody function. Excess lambda or kappa light chains (Bence Jones proteins, BJP) are commonly produced by the myeloma cells. BJP's are present in the cytoplasm of the cells and are also secreted into the blood. They are frequently secreted as monomer (22-25 kD) and dimer (50 kD) forms and are small enough to pass freely into the urine (Durie (2003) International Myeloma Foundation, Multiple Myeloma, Concise Review of the Disease and Treatment Options).

The presence of membrane bound lambda light chain that is not associated with heavy chain would provide a novel therapeutic target on myeloma cells.

Summary of the Invention

The present inventors have now identified a new target on myeloma cells for use in methods designed for the diagnosis or treatment of multiple myeloma and other lymphoproliferative disorders. This target is the lambda myeloma antigen (LMA).

The therapeutic methods proposed by the present inventors are based on the administration of a binding moiety or ligand that binds specifically to LMA for the depletion of malignant cells in patients suffering from lymphoproliferative disorders. Preferably, the ligand is an anti-LMA antibody. The therapeutic approaches described herein represent a radical departure from previous and currently available treatments for lymphoproliferative disorders.

Accordingly, in one aspect the present invention provides a method for the treatment of a lymphoproliferative disorder in a subject, the method comprising administering to the subject an effective amount of an anti-LMA antibody.

The present invention also provides the use of an anti-LMA antibody for the preparation of a medicament for the treatment of a lymphoproliferative disorder.

In a preferred embodiment of the invention, the lymphoproliferative disorder is selected from the group consisting of multiple myeloma, B cell lymphoma and macroglobulinemia. Preferably, the lymphoproliferative disorder is multiple myeloma, and more preferably lambda-type multiple myeloma.

In one particular aspect the present invention is directed to a method of inhibiting the growth of, or killing, myeloma cells in a patient by administering an anti-LMA antibody under conditions sufficient for the binding of the antibody to the myeloma cells to cause inhibition or killing of the cancer cells. Preferably, the inhibition or killing of the cancer cells is effected by apoptosis or by the immune cells of the patient.

In another aspect, a method for inhibiting or killing myeloma cells in a patient is provided by administering an LMA ligand which is conjugated with a cytotoxic moiety or biological modifier, under conditions sufficient for the binding of the monoclonal antibody to the cancer cells to inhibit the growth of, or to kill, the cells.

In one embodiment, the cytotoxic moiety is a toxin (which may be a photo-activated toxin), a chemotherapeutic agent, or a radioactive agent.

By way of non-limiting examples, the cytotoxic moiety of the immunotoxin may be a cytotoxic drug or an enzymatically active toxin of bacterial or plant origin (such as gelonin), or an enzymatically active fragment ("A chain") of such a toxin. Enzymatically active toxins and fragments thereof are preferred and are exemplified by gelonin, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolacca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, saponaria officinalis inhibitor, mitogellin, restrictocin, phenomycin, and enomycin.

Cytotoxic drugs which are useful in the present invention include, but are not limited to, adriamycin (and derivatives thereof), cis-platinum complex (and derivatives thereof), bleomycin and methotrexate (and derivatives thereof). These cytotoxic drugs are sometimes useful for clinical management of recurrent tumors and particularly breast cancer, but their use is complicated by severe side effects and damage caused to non-target cells. Anti-LMA antibodies may serve as a useful carrier of such drugs providing an efficient means of both delivery to the cancer cells and enhanced entry into the cancer cells themselves.

Biological response modifiers which may be coupled to the anti-LMA antibody and used in the present invention include, but are not limited to, lymphokines and cytokines such as IL-2 and interferons (α, β or γ). These biological response modifiers have a variety of effects on tumor cells. Among these effects are increased tumor cell killing by direct action as well as increased tumor cell killing by increased host defence mediated processes. Conjugation of an anti-LMA antibody to these biological response modifiers will allow selective localization within tumors and, hence, improved anti-proliferative effects while suppressing non-specific effects leading to toxicity of non-target cells.

Conjugates of the anti-LMA antibody may be made using a variety of bifunctional protein coupling agents. Examples of such reagents are SPDP, IT, bifunctional derivatives of imidoesters such as dimethyl adipimidate HCl, active esters such as disuccinimidyl suberate, aldehydes such as glutaraldehyde, bis-azido compounds such

as bis(p-azidobenzoyl) hexanediamine, bis-diazonium derivatives such as bis-(p-diazoniumbenzoyl)-ethylenediamine, diisocyanates such as tolylene 2,6-diisocyanate, and bis-active fluorine compounds such as a 1,5-difluoro-2,4-dinitrobenzene.

Cytotoxic radiopharmaceuticals for diagnosing and treating cancer cells carrying the LMA antigen may be made by conjugating high linear energy transfer (LET) emitting isotopes (e.g., Y, Pr) to the antibodies. The term "cytotoxic moiety" as used herein is intended to include such isotopes.

The labels that are used in making labeled versions of the antibodies include moieties that may be detected directly, such as fluorochromes and radiolabels as well as moieties, such as enzymes, that must be reacted or derivatized to be detected. Examples of such labels are ^{32}P, ^{125}I, ^{3}H, ^{14}C, fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferia, 2,3-dihydrophthalzainediones, horseradish peroxidase, alkaline phosphatase, lysozyme, and glucose-6-phosphate dehydrogenase. The antibodies may be tagged with such labels by known methods. For instance, coupling agents such as aldehydes, carbodiimides, dimaleimide, imidates, succinimides, bis-diazotized benzadine and the like may be used to couple the antibodies with the above-described fluorescent, chemiluminescent, and enzyme labels.

In another embodiment the cytotoxic moiety is a nucleic acid molecule encoding a cytotoxic agent. In this embodiment, the anti-LMA antibody functions as a carrier to introduce a therapeutic gene encoding a cytotoxic agent, e.g., toxin genes such as diphtheria toxin-A, lectins, Pseudomonas exotoxin A, Saponaria officinalis SO-6 (Soria, Pharma. Res., 21, 35 (1989)) or ricin; cell suicide genes such as thynidine kinase or nitroreductase; proteins that activate chemotherapeutic genes such as gangcyclovir or mitomycin C; a ribozyme, RNase, or an antisense sequence (e.g., BCL2 sequence); into LMA+ cells such as myeloma cells. Preferably, the expression of only a few molecules of the cytotoxic agent encoded by the therapeutic gene are sufficient to kill a cell that expresses that gene. It is preferred that the therapeutic gene is operatively linked to a cell or tissue-specific transcription unit, e.g., a cell or tissue-specific promoter and/or enhancer. Preferred transcription units are those which direct expression in B cells (e.g., transcription units from an Ig heavy gene, Ig kappa gene, Ig lambda gene, BCL-6 gene (Dalla Favera *et al.*, C.S.H. Smp. Quant. Biol., 59, 117 (1994)), CD19 gene, CD20 gene, or CD22 gene (Kerhl *et al.*, Immunol. Today, 15, 432 (1994)), T cells (e.g., transcription units from the IL-4 gene, IL-2 gene, IL-2R gene, T

cell receptor gene, IL-5 gene, IL-13 gene, GM-CSF gene and Fas ligand gene (Nagata *et al.*, Prog. Mol. Subcell. Biol., 16, 87 (1996)) or myeloid cells. Myeloid-specific transcription units include, but are not limited to, those disclosed in U.S. Pat. No. 5,502,176, as well as transcription units from the PU.1 gene (Fisher *et al.*, Stem Cells, 16, 25 (1998)), CD11c or CD18 gene (Corbi *et al.*, Leuk. & Lymph., 25, 415 (1997)), IgH enhancer, CSF receptor G, GM and/or G gene (Zhang *et al.*, Cur. Top. Micro. & Immunol., 211, 137 (1996)), or the C/EBP, Runt/PEBP2/CBF or Ets gene (Clarke *et al.*, J. Leuko. Biol., 63, 153 (1998)).

In a preferred embodiment the method of the present invention further comprises the step of treating the subject to reduce the levels of free lambda light chains present in the fluid of the subject prior to administration of the anti-LMA antibody or fusion polypeptide. Preferably, the levels of free light chains present in the serum of the subject are reduced. A reduction in the levels of free light chains may be achieved by, for example, chemotherapy or plasmapheresis. It is preferred that the treatment for reducing levels of free light chains is performed on the subject just prior to administration of the anti-LMA antibody or fusion polypeptide.

In a further aspect of the invention, a method is provided for removing myeloma cells from an isolated cellular sample, such as, but not limited to, bone marrow cells, by exposing the cellular sample to a an anti-LMA antibody or LMA ligand/cytotoxin conjugate under conditions wherein the myeloma cells bind to the antibody or conjugate, and isolating a cellular fraction of said cellular sample which does not bind to the antibody or conjugate. This method may be used, for example, in the removal of myeloma cells from a bone marrow sample for autologous bone marrow transplant.

Thus, the present invention also provides a method for autologous hematopoietic cell transplantation in a subject, the method comprising

(i) removing a hematopoietic progenitor cell population from the subject,

(ii) treating the cell population with an anti-LMA antibody or LMA ligand/cytotoxin , and

(iii) transplanting the treated cell population from step (ii) into the subject.

In a preferred embodiment of this aspect, the method also involves intravenous infusion of anti-LMA antibody or LMA ligand/cytotoxin conjugate into the subject.

In yet a further preferred embodiment of this, the method of autologous transplantation is performed on the subject during or after cytoreductive therapy.

In yet a further preferred embodiment of this aspect, the anti-LMA antibody or LMA ligand/cytotoxin conjugate is bound to a solid support.

The invention is also directed to an anti-LMA antibody or LMA ligand/cytotoxin bound to a solid support.

In still another aspect of the invention, the above-mentioned conjugate or the anti-LMA antibody may be used *in vitro* to inhibit growth of, or kill, myeloma cells in a cellular sample, such as a bone marrow sample.

The invention is also directed to anti-idiotypic antibodies which mirror the binding site of an anti-LMA antibody, and are specific to the myeloma conformational epitope recognized by the antibody. The invention is further directed to the use of these anti-idiotypic antibodies for the treatment of multiple myeloma by active immunization.

In yet another aspect of the invention, a method is provided for localizing cancer cells in a patient by administering an anti-LMA antibody or LMA ligand, allowing the antibody or ligand to bind to the cancer cells within said patient, and determining the location of the antibody within the patient. In a preferred embodiment of this aspect, the antibody or ligand is detectably labeled, for example, with a radionuclide, a fluorophore, a chromophore or an enzyme.

In another aspect the present invention provides an anti-LMA antibody conjugated to a cytotoxic moiety. In one embodiment of this aspect, the cytotoxic moiety is a toxin, a photo-activated toxin, a chemotherapeutic agent, or a radioactive agent.

In another embodiment of this aspect, the cytotoxic moiety is a nucleic acid molecule. Thus, the invention provides a therapeutic composition which selectively targets LMA cell surface molecules but has reduced or no immunogenicity as the therapeutic gene, preferably in the form of circular DNA such as plasmid DNA, rather than an immunogenic protein, is introduced to the host mammal. As a result, it may be possible to repeatedly administer the therapeutic composition to a mammal, e.g., myeloma patients, without the development of significant antibody responses, particularly to the

cytotoxic agent encoded by the therapeutic gene. Moreover, a therapeutic composition of the invention is useful to kill cells in patients with other LMA+ plasmaproliferative disorders such as B cell lymphoma and macroglobulinemia. Preferably, a humanized version of the antibody portion of the fusion polypeptide in the composition is employed for use in humans.

In another aspect, the present invention is directed to an anti-LMA antibody labeled with a detectable moiety, such as, by way of non-limiting examples, a fluorophore, a chromophore, a radionuclide, or an enzyme.

In still yet another aspect, the invention is directed to a pharmaceutical composition comprising an anti-LMA antibody as described above and a pharmaceutically-acceptable carrier, diluent, or excipient.

In a further preferred embodiment of the present invention, the anti-LMA antibody is a chimeric antibody or a humanised antibody.

Brief description of the Figures

Figure 1. Analysis of the specificity of L7 using and ELISA. The mAb, L7, was incubated on an ELISA plate coated with human lambda light chain antigens (Lam F, Lam H, MOS), pooled normal human IgG (λ and κ) and human kappa light chain (VOR). Bound antibody was detected with an anti-mouse IgG-AP conjugate. Antibody-antigen complexes were visualized by colour development and absorbance measured at 405 nm.

Figure 2. Binding of L7 to LP-1 myeloma cells in the presence and absence of soluble antigen. LP-1 myeloma cells (5×10^5) were incubated with L7 (100μg/mL) alone (solid black line) or with L7 pre-incubated with soluble antigen. Pre-incubation was performed with free lambda light chains, λFLC (thin black line) or free kappa light chains, κLC (solid grey line). Cells were then washed twice and incubated with PE-labelled goat-anti-mouse $F(ab')_2$, washed and analysed by flow cytometry. The solid grey histogram represents cells which were incubated with PBS followed by the PE-labelled goat-anti-mouse $F(ab')_2$.

Figure 3. Effect of antigen binding site occupancy on the binding of L7 to LP-1 cells. LP-1 myeloma cells (5×10^5) were incubated with L7 (100μg/mL) pre-incubated with free kappa light chains, κLC (solid grey line) or with L7 pre-incubated with a range of free lambda light chain concentrations, λFLC. Cells were then washed twice and incubated with PE-labelled goat-anti-mouse $F(ab')_2$, washed and analysed by flow cytometry. The solid grey histogram represents cells which were incubated with PBS followed by the PE-labelled goat-anti-mouse $F(ab')_2$.

Detailed Description of the Invention

General Techniques

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel *et al.* (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan *et al.* (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present), and are incorporated herein by reference.

Definitions

The term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass

known analogs of natural nucleotides that can function in a manner similar to naturally occurring nucleotides.

The phrase "nucleic acid encoding" or "nucleic acid sequence encoding" refers to a nucleic acid, i.e., DNA or RNA, which directs the expression of a specific polypeptide, protein or peptide. The nucleic acid sequences include both the DNA strand sequence that is transcribed into RNA and the RNA sequence that is translated into protein. The nucleic acid sequences include both full length nucleic acid sequences as well as shorter sequences derived from the full length sequences. It is understood that a particular nucleic acid sequence includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell. The nucleic acid includes both the sense and antisense strands as either individual single strands or in the duplex form.

The terms "isolated" or "substantially purified," when referring to recombinantly produced polypeptides, or DNA encoding a cytotoxic agent of the invention, means a chemical composition which is essentially free of other cellular components. Such a composition is preferably in a homogeneous state although it can be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography (for polypeptides), or A_{260}/A_{280} ratios (for nucleic acids). A polypeptide which is the predominant species present in a preparation is substantially purified. Generally, a substantially purified or isolated polypeptide or nucleic acid molecule comprises more than 80% of all macromolecular species present in the preparation. Preferably, the polypeptide or nucleic acid molecule is purified to represent greater than 90% of all macromolecular species present. More preferably the polypeptide or nucleic acid molecule is purified to greater than 95%, and most preferably the polypeptide or nucleic acid molecule is purified to essential homogeneity, wherein other macromolecular species are not detected by conventional techniques.

As used herein "ligands" or "binding moieties" are molecules capable of reacting with or otherwise recognizing and specifically binding LMA. The binding between a ligand and its target may be mediated by covalent or non-covalent interactions or a combination of covalent and non-covalent interactions. When the interaction of the two species produces a non-covalently bound complex, the binding which occurs is

typically electrostatic, hydrogen-bonding, or the result of hydrophilic/lipophilic interactions. Accordingly, "specific binding" occurs between a ligand and LMA where there is interaction between the two which produces a bound complex having the characteristics of an antibody/antigen or enzyme/substrate interaction. Specifically, examples of ligands include, but are not limited to antibodies and the like which specifically bind desired target cells.

The phrase "binding specificity," or "specifically immunoreactive with," refers to a binding reaction which is determinative of the presence of a protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under particular conditions, the fusion polypeptides of the invention bind to a particular protein, i.e., LMA, and do not bind in a significant amount to other proteins or carbohydrates present in the sample. Specific binding to LMA under such conditions may require an antibody that is selected for its specificity for a particular protein or carbohydrate. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein or carbohydrate. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein or carbohydrate. See Harlow and Lane (1988) Antibodies, a Laboratory Manual, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

The terms "recombinant DNA," "recombinant nucleic acid" or "recombinantly produced DNA" refer to DNA which has been isolated from its native or endogenous source and modified either chemically or enzymatically by adding, deleting or altering naturally occurring flanking or internal nucleotides. Flanking nucleotides are those nucleotides which are either upstream or downstream from the described sequence or sub-sequence of nucleotides, while internal nucleotides are those nucleotides which occur within the described sequence or sub-sequence.

The term "labeled antibody" as used herein refers to an antibody bound to a label such that detection of the presence of the label (e.g., as bound to a biological sample) indicates the presence of the antibody.

A "cytotoxic moiety" refers to a molecule that when contacted with a cell brings about the death of that cell.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

LMA Binding Moieties

The present inventors have now shown, for the first time, that LMA is expressed on the surface of myeloma cells. It is envisaged that binding moieties, particularly antibodies, directed against LMA will be capable of killing lambda-type myeloma cells through mechanisms such as ADCC, complement dependent lysis and apoptosis and will therefore be effective therapeutic agents against lambda-type myeloma cells. In addition, binding moieties directed against LMA can be used to deliver cytotoxins directly to malignant cells.

When used herein, the phrase "LMA binding moiety" refers to a moiety that specifically recognises the membrane bound and free forms of LMA of approximately 26kD in the monomeric form and approximately 52kD in the dimeric form. By "free" LMA we mean LMA that is not associated with an intact immunoglobulin.

LMA will be known to those skilled in the art. For example, antibodies directed against LMA have been used to detect free lambda light chains in serum or urine in tests for diagnosing multiple myeloma (Bradwell *et al.*, 2001). LMA has not been used to date, however, as a target for the treatment of multiple myeloma or for the localization of myeloma cells in a patient.

Preferred LMA binding moieties are polypeptides or compounds identified as having binding affinity to LMA. Preferred LMA binding moieties are anti-LMA antibodies (naturally occurring or recombinant, from any source), e.g., RDI-TRK1L7-3D12, (RDI; Flanders, NJ, USA), CBL317 (Cymbus Biotechnology Ltd, UK) and 2G7 (Nakano and Nagata (2003) J Immunol Methods 275; 9-17).

As used herein, the term "antibody" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa and

lambda light chain genes, and alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad of immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

The basic immunoglobulin (antibody) structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

Antibodies may exist as intact immunoglobulins, or as modifications in a variety of forms including, for example, $FabFc_2$, Fab, Fv, Fd, $(Fab')_2$, an Fv fragment containing only the light and heavy chain variable regions, a Fab or (Fab)'2 fragment containing the variable regions and parts of the constant regions, a single-chain antibody (Bird *et al.*, Science, 242: 424-426 (1988); Huston *et al.*, Proc. Natl. Acad. Sci. USA, 85: 5879-5883 (1988) both incorporated by reference herein), CDR-grafted antibodies and the like. The heavy and light chain components of an Fv may be derived from the same antibody or different antibodies thereby producing a chimeric Fv region. The antibody may be of animal (especially mouse or rat) or human origin or may be chimeric (Morrison *et al.*, Proc. Natl. Acad. Sci. USA, 81, 6851-6855 (1984) both incorporated by reference herein) or humanized (Jones *et al.*, Nature, 321, 522-525 (1986), and published UK patent application #8707252, both incorporated by reference herein). As used herein the term "antibody" includes these various forms. Using the guidelines provided herein and those methods well known to those skilled in the art which are described in the references cited above and in such publications as Harlow & Lane, Antibodies: a Laboratory Manual, Cold Spring Harbor Laboratory, (1988) the antibodies of the present invention can be readily made.

The LMA-binding antibodies may be Fv regions comprising a variable light (V_L) and a variable heavy (V_H) chain. The light and heavy chains may be joined directly or through a linker. As used herein a linker refers to a molecule that is covalently linked to the light and heavy chain and provides enough spacing and flexibility between the two chains such that they are able to achieve a conformation in which they are capable

of specifically binding the epitope to which they are directed: Protein linkers are particularly preferred as they may be expressed as an intrinsic component of the Ig portion of the fusion polypeptide.

Another preferred embodiment of the invention is a recombinantly produced single chain scFv antibody, preferably a humanized scFv.

Preparation of Genes Encoding Antibodies or Fragments Thereof

Genes encoding antibodies, both light and heavy chain genes or portions thereof, e.g., single chain Fv regions, may be cloned from a hybridoma cell line. They may all be cloned using the same general strategy. Typically, for example, poly(A)$^+$mRNA extracted from the hybridoma cells is reverse transcribed using random hexamers as primers. For Fv regions, the V_H and V_L domains are amplified separately by two polymerase chain reactions (PCR). Heavy chain sequences may be amplified using 5' end primers which are designed according to the amino-terminal protein sequences of the anti-LMA heavy chains respectively and 3' end primers according to consensus immunoglobulin constant region sequences (Kabat *et al.*, Sequences of Proteins of Immunological Interest. 5th edition. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, Md. (1991) incorporated by reference). Light chain Fv regions are amplified using 5' end primers designed according to the amino-terminal protein sequences of anti-LMA light chains and in combination with the primer C-kappa. One of skill in the art would recognize that many suitable primers may be employed to obtain Fv regions.

The PCR products are subcloned into a suitable cloning vector. Clones containing the correct size insert by DNA restriction are identified. The nucleotide sequence of the heavy or light chain coding regions may then be determined from double stranded plasmid DNA using sequencing primers adjacent to the cloning site. Commercially available kits (e.g., the Sequenase.TM. kit, United States Biochemical Corp., Cleveland, Ohio, USA) may be used to facilitate sequencing the DNA.

Thus, DNA encoding the Fv regions may be prepared by any suitable method, including, for example, amplification techniques such as ligase chain reaction (LCR) (see Wu and Wallace, Genomics, 4: 560 (1989), Landegren, *et al.*, Science, 241: 1077 (1988) and Barringer, *et al.*, Gene, 89: 117 (1990)), transcription amplification (see

Kwoh, *et al.*, Proc. Natl. Acad. Sci. USA, 86: 1173 (1989)), and self-sustained sequence replication (see Guatelli, *et al.*, Proc. Natl. Acad. Sci. USA, 87: 1874 (1990)), cloning and restriction of appropriate sequences or direct chemical synthesis by methods such as the phosphotriester method of Narang *et al.*, Meth. Enzymol. 68: 90-99 (1979); the phosphodiester method of Brown *et al.*, Meth Enzymol. 68: 109-151 (1979); the diethylphosphoramidite method of Beaucage *et al.*, Tetra. Lett., 22: 1859-1862 (1981); and the solid support method of U.S. Pat. No. 4,458,066, all such references in this paragraph incorporated by reference herein.

Chemical synthesis produces a single stranded oligonucleotide. This may be converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. While it is possible to chemically synthesize an entire single chain Fv region, it is preferable to synthesize a number of shorter sequences (about 100 to 150 bases) that are later ligated together.

Alternatively, sub-sequences may be cloned and the appropriate subsequences cleaved using appropriate restriction enzymes. The fragments may then be ligated to produce the desired DNA sequence.

Once the Fv variable light and heavy chain DNA is obtained, the sequences may be ligated together, either directly or through a DNA sequence encoding a peptide linker, using techniques well known to those of skill in the art. In one embodiment, heavy and light chain regions are connected by a flexible peptide linker (e.g., $(Gly_4Ser)_3$) which starts at the carboxyl end of the heavy chain Fv domain and ends at the amino terminus of the light chain Fv domain. The entire sequence encodes the Fv domain in the form of a single-chain antigen binding protein.

Cytotoxic moieties

Suitable cytotoxic moieties for use in the present invention includes, but is not limited to, agents such as bacterial or plant toxins, drugs, e.g., cyclophosphamide (CTX; cytoxan), chlorambucil (CHL; leukeran), cisplatin (CisP; CDDP; platinol), busulfan (myleran), melphalan, carmustine (BCNU), streptozotocin, triethylenemelamine (TEM), mitomycin C, and other alkylating agents; methotrexate (MTX), etoposide (VP-16; vepesid), 6-mercaptopurine (6MP), 6-thioguanine (6TG), cytarabine (Ara-C),

5-fluorouracil (5FU), dacarbazine (DTIC), 2-chlorodeoxyadenosine (2-CdA), and other antimetabolites; antibiotics including actinomycin D, doxorubicin (DXR; adriamycin), daunorubicin (daunomycin), bleomycin, mithramycin as well as other antibiotics; alkaloids such as vincristin (VCR), vinblastine, and the like; as well as other anti-cancer agents including the cytostatic agents glucocorticoids such as dexamethasone (DEX; decadron) and corticosteroids such as prednisone, nucleotide enzyme inhibitors such as hydroxyurea, and the like.

Those skilled in the art will realize that there are numerous other radioisotopes and chemocytotoxic agents that can be coupled to tumor specific antibodies by well known techniques, and delivered to specifically destroy tumor tissue. See, e.g., U.S. Pat. No. 4,542,225 to Blattler *et al.* Examples of photo-activated toxins include dihydropyridine- and omega-conotoxin (Schmidt *et al.*, J Biol. Chem., 1991, 266(27):18025-33). Examples of imaging and cytotoxic reagents that can be used include ^{125}I, ^{111}In, ^{123}I, ^{99}mTc, ^{32}P, ^{3}H, and ^{14}C; fluorescent labels such as fluorescein and rhodamine, and chemiluminescers such as luciferin. The antibody can be labeled with such reagents using techniques known in the art. For example, see Wenzel and Meares, Radioimmunoimaging and Radioimmunotherapy, Elsevier, N.Y. (1983) for techniques relating to the radiolabeling of antibodies (see also, Colcer *et al.*, "Use of Monoclonal Antibodies As Radiopharmaceuticals For The Localization Of Human Carcinoma Xenografts In Nude Mice", Methods Enzymol., 121:802-16, 1986: "Order, Analysis, Results and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy", in Monoclonal Antibodies for Cancer Detection and Therapy, Baldwin *et al.* (eds), pp. 303-16 (Academic Press 1985).

When a DNA molecule encoding a cytotoxic agent is present in a therapeutic composition of the invention, the DNA preferably encodes a polypeptide that is a bacterial or plant toxin. These polypeptides include, but are not limited to, polypeptides such as native or modified Pseudomonas exotoxin (PE), diphtheria toxin (DT), ricin, abrin, gelonin, momordin II, bacterial RIPs such as shiga and shiga-like toxin a-chains, luffin [see Islam *et al.*, Agricultural Biological Chem., 54(5):1343-1345 (1990)], atrichosanthin [see Chow *et al.*, J. Biol. Chem., 265:8670-8674 (1990))], momordin I [see Ho et al., BBA, 1088:311-314 (1991)], Mirabilis anti-viral protein [see Habuka et al., J. Biol. Chem., 264(12):6629-6637 (1989)], pokeweed antiviral protein [see Kung et al., Agric. Biol. Chem., 54(12):3301-3318 (1990)], byodin 2 (U.S. Pat. No. 5,597,569), gaporin [see Benatti et al., Eur. J. Biochem., 183:465-470 (1989)],

as well as genetically engineered variants thereof. Native PE and DT are highly toxic compounds that typically bring about death through liver toxicity. Preferably, PE and DT are modified into a form that removes the native targeting component of the toxin, e.g., domain Ia of PE and the B chain of DT. One of skill in the art will appreciate that the invention is not limited to a particular cytotoxic agent.

The term "Pseudomonas exotoxin" (PE) as used herein refers to a full-length native (naturally occurring) PE or a PE that has been modified. Such modifications may include, but are not limited to, elimination of domain Ia, various amino acid deletions in domains II and III, single amino acid substitutions (e.g., replacing Lys with Gln at positions 590 and 606), and the addition of one or more sequences at the carboxyl terminus. See Siegall *et al.*, J. Biol. Chem., 264: 14256-14261 (1989). Thus, for example, PE38 refers to a truncated Pseudomonas exotoxin composed of amino acids 253-364 and 381-613. The native C-terminus of PE, REDLK (residues 609-613), may be replaced with the sequence KDEL, REDL, and Lys-590 and Lys-606 may be each mutated to Gln.

The term "Diphtheria toxin" (DT) as used herein refers to full length native DT or to a DT that has been modified. Modifications typically include removal of the targeting domain in the B chain and, more specifically, involve truncations of the carboxyl region of the B chain.

Preparation of Antibody Fusion Polypeptides

Once a DNA sequence has been identified that encodes an LMA binding moiety (eg. an anti-LMA antibody fragment) fusion polypeptides comprising that region may be prepared by methods known to one of skill in the art. For example, a gene encoding an Fv region is fused to a gene encoding a cytotoxic moiety, preferably a moiety which is a polypeptide. Optionally, the Fv gene is linked to a segment encoding a peptide connector. The peptide connector may be present simply to provide space between the LMA binding moiety and the cytotoxic moiety or to facilitate mobility between these regions to enable them to each attain their optimum conformation. The DNA sequence comprising the connector may also provide sequences (such as primer sites or restriction sites) to facilitate cloning or may preserve the reading frame between the sequence encoding the binding moiety and the sequence encoding the cytotoxic moiety. The design of such connector peptides is well known to those of skill in the art.

Generally producing fusion polypeptides involves separately preparing the Fv light and heavy chains and DNA encoding any other protein to which they are fused and recombining the DNA sequences in a plasmid or other vector to form a construct encoding the particular desired fusion polypeptide. However, a simpler approach involves inserting the DNA encoding the particular Fv region into a construct already encoding the desired second polypeptide. The DNA sequence encoding the Fv region is inserted into the construct using techniques well known to those of skill in the art.

One embodiment of the invention is a fusion polypeptide comprising a recombinantly produced antibody comprising a V_H and C_H, or a portion thereof, joined to a DNA binding polypeptide. The fusion polypeptide and an antibody comprising V_L and C_L, or a portion thereof, together form a recombinant antibody useful to direct preselected DNA molecules, either linear or circular, to a cell or tissue bearing the preselected target molecule.

Another preferred embodiment of the invention is a recombinantly produced single chain scFv antibody, preferably a humanized scFv. In particular, this invention provides for recombinant single chain antibodies that are joined to a DNA binding polypeptide and, because of their ability to specifically bind to DNA, these antibodies are useful as targeting moieties which serve to direct DNA which is bound to DNA binding polypeptide to a cell or tissue bearing LMA.

The recombinant single chain antibodies of the present invention may be fused to, or otherwise bound to the cytotoxin or other molecule having a specified activity by any method known and available to those in the art. The two components may be chemically bonded together by any of a variety of well-known chemical procedures. For example, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins, as well as chemical conjugation methods, are well-known within the art and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe *et al.*, Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982); Waldmann, Science, 252: 1657 (1991); Vitetta *et al.*, 1987, Science, 238:1098; Pastan *et al.*, 1986; Cell, 47:641; and Thorpe *et al.*, 1987, Cancer Res., 47:5924, which are incorporated by reference herein. These methods generally conjugate the cytotoxin and the antibody by means of cross-linking agents that

introduce a disulfide bond between the two polypeptides. Immunotoxins which have been prepared with nonreducible linkages have been shown to be consistently less cytotoxic than similar toxins cross-linked by disulfide bonds.

Other preferred reagents are 2-iminothiolane hydrochloride (2IT), sodium S-4-succinimidyloxycarbonyl-.alpha.-methyl benzyl thiosulfate (SMBT) and 2IT or succinimidyloxy carbonyl-α-methyl-α(2-pyrid- yldithio)-toluene and 2IT. Each group of reagents introduces a disulfide bond between the cytotoxin and the antibody which is reducible, but the bond is also resistant to breakdown providing stability of the conjugate *in vitro* and *in vivo*. Upon internalization into lysosomes or endosomes by the target cell, the bond is reduced. For example, to use the recombinant PE molecules with an antibody, a form of the PE molecule with cysteine at amino acid position 287 is preferred to couple the toxin to the antibody or other ligand through the thiol moiety of cysteine.

In one embodiment, the LMA binding moiety may also be fused to a cytotoxin by recombinant means such as through the use of recombinant DNA techniques to produce a nucleic acid which encodes both the antibody and the DNA binding polypeptide and expressing the recombinant DNA sequence in a host cell, such as a eukaryotic, e.g., mammalian such as CHO or COS cells, or prokaryotic, e.g., *E. coli*, host. The DNA encoding the fusion polypeptide may be cloned in cDNA or in genomic form by any cloning procedure known to those skilled in the art. See for example Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory, (1989), which is herein incorporated by reference.

One of skill in the art would recognize that after chemical synthesis, biological expression, or purification, a fusion polypeptide may possess a conformation substantially different than the native antibody. In this case, it may be necessary to denature and reduce the polypeptide and then to cause the polypeptide to re-fold into the preferred conformation. Methods of reducing and denaturing the polypeptide and inducing re-folding are well known to those of skill in the art. (See, Debinski *et al.*, J. Biol. Chem, 268: 14065-14070 (1993); Kreitman and Pastan, Bioconjug. Chem., 4: 581-585 (1993); and Buchner, *et al.*, Anal. Biochem., 205: 263-270 (1992) which are incorporated herein by reference.) Debinski *et al.*, for example, describe the denaturation and reduction of inclusion body proteins in guanidine-DTE. The

polypeptide is then refolded in a redox buffer containing oxidized glutathione and L-arginine.

One of skill would recognize that modifications can be made to the fusion polypeptides without diminishing their biological activity. Some modifications may be made to facilitate the cloning, expression, or incorporation of the antibody portion of the fusion polypeptide into the fusion polypeptide. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, or additional amino acids placed on either terminus to create conveniently located restriction sites or termination codons.

One of skill will recognize that other modifications may be made. Thus, for example, amino acid substitutions may be made that increase specificity or binding affinity of the fusion polypeptide. Alternatively, non-essential regions of the molecule may be shortened or eliminated entirely. Thus, where there are regions of the molecule that are not themselves involved in the activity of the molecule, they may be eliminated or replaced with shorter segments that serve to maintain the correct spatial relationships between the active components of the molecule. Alternatively more flexible segments may be placed in interdomain regions which then can facilitate folding or production of the molecule (Brinkmann, *et al.*, Proc. Natl. Acad. Sci. USA, 89: 3075-3079 (1992).

Monoclonal antibodies

Monoclonal antibodies directed against LMA epitopes can be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Panels of monoclonal antibodies produced against LMA epitopes can be screened for various properties; i.e. for isotype and epitope affinity.

Mouse-derived monoclonal antibodies can be used for both direct *in vivo* and extracorporeal immunotherapy. However, it has been observed that when mouse-derived monoclonal antibodies are used in humans as therapeutic agents, the patient produces human anti-mouse antibodies. Thus, mouse-derived monoclonal antibodies are not preferred for therapy, especially for long term use. With established genetic

engineering techniques it is possible, however, to create chimeric or humanized antibodies that have animal-derived and human-derived portions. The animal can be a mouse or another rodent such as a rat.

If the variable region of the chimeric antibody is mouse-derived while the constant region is human-derived, the chimeric antibody will generally be less immunogenic than a "pure" mouse-derived monoclonal antibody. These chimeric antibodies would likely be more suited for therapeutic use, should it turn out that "pure" mouse-derived antibodies are unsuitable.

Chimeric Antibodies

Methodologies for generating chimeric antibodies are available to those in the art. For example, the light and heavy chains can be expressed separately, using, for example, immunoglobulin light chain and immunoglobulin heavy chains in separate plasmids. These can then be purified and assembled *in vitro* into complete antibodies; methodologies for accomplishing such assembly have been described. See, for example, Scharff, M., Harvey Lectures 69:125 (1974). See also Oi *et al.*, Bio Techniques 4(4):214-221 (1986); and Sun *et al.* Hybridoma 5 (1986) Suppl 1:517-20. Such a DNA construct may comprise DNA encoding functionally rearranged genes for the variable region of a light or heavy chain of an anti-LMA antibody linked to DNA encoding a human constant region. Lymphoid cells such as myelomas or hybridomas transfected with the DNA constructs for light and heavy chain can express and assemble the antibody chains.

In vitro reaction parameters for the formation of IgG antibodies from reduced isolated light and heavy chains have also been described. See, for example, Beychok, S., Cells of Immunoglobulin Synthesis, Academic Press, New York, p. 69, 1979. Co-expression of light and heavy chains in the same cells to achieve intracellular association and linkage of heavy and light chains into complete H2L2 IgG antibodies is also possible. Such co-expression can be accomplished using either the same or different plasmids in the same host cell.

Humanised antibodies

In another preferred embodiment of the present invention the anti-LMA antibody is humanised, that is, an antibody produced by molecular modeling techniques wherein the human content of the antibody is maximised while causing little or no loss of binding affinity attributable to the variable region of the murine antibody.

The method described below are applicable to the humanisation of anti-LMA antibodies.

There are several factors to consider in deciding which human antibody sequence to use during the humanisation. The humanisation of light and heavy chains are considered independently of one another, but the reasoning is basically similar for each.

This selection process is based on the following rationale: A given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable domain framework residues have little or no direct contribution. The primary function of the framework regions is to hold the CDRs in their proper spatial orientation to recognize antigen. Thus the substitution of rodent CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework is highly homologous to the rodent variable domain from which they originated. A human variable domain should preferably be chosen therefore that is highly homologous to the rodent variable domain(s). A suitable human antibody variable domain sequence can be selected as follow.

Step 1. Using a computer program, search all available protein (and DNA) databases for those human antibody variable domain sequences that are most homologous to the rodent antibody variable domains. The output of a suitable program is a list of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each sequence to the rodent sequence. This is done independently for both the heavy and light chain variable domain sequences. The above analyses are more easily accomplished if only human immunoglobulin sequences are included.

Step 2. List the human antibody variable domain sequences and compare for homology. Primarily the comparison is performed on length of CDRs, except CDR3 of the heavy chain which is quite variable. Human heavy chains and Kappa and Lambda light chains are divided into subgroups; Heavy chain 3 subgroups, Kappa chain 4 subgroups, Lambda chain 6 subgroups. The CDR sizes within each subgroup are similar but vary between subgroups. It is usually possible to match a rodent antibody CDR to one of the human subgroups as a first approximation of homology. Antibodies bearing CDRs of similar length are then compared for amino acid sequence homology, especially within the CDRs, but also in the surrounding framework regions. The human variable domain which is most homologous is chosen as the framework for humanisation.

The Actual Humanising Methodologies/Techniques

An antibody may be humanised by grafting the desired CDRs onto a human framework according to EP-A-0239400. A DNA sequence encoding the desired reshaped antibody can therefore be made beginning with the human DNA whose CDRs it is wished to reshape. The rodent variable domain amino acid sequence containing the desired CDRs is compared to that of the chosen human antibody variable domain sequence. The residues in the human variable domain are marked that need to be changed to the corresponding residue in the rodent to make the human variable region incorporate the rodent CDRs. There may also be residues that need substituting in, adding to or deleting from the human sequence.

Oligonucleotides are synthesized that can be used to mutagenize the human variable domain framework to contain the desired residues. Those oligonucleotides can be of any convenient size. One is normally only limited in length by the capabilities of the particular synthesizer one has available. The method of oligonucleotide-directed in vitro mutagenesis is well known.

Alternatively, humanisation may be achieved using the recombinant polymerase chain reaction (PCR) methodology of WO 92/07075. Using this methodology, a CDR may be spliced between the framework regions of a human antibody.

In general, the technique of WO 92/07075 can be performed using a template comprising two human framework regions, AB and CD, and between them, the CDR

which is to be replaced by a donor CDR. Primers A and B are used to amplify the framework region AB, and primers C and D used to amplify the framework region CD. However, the primers B and C each also contain, at their 5' ends, an additional sequence corresponding to all or at least part of the donor CDR sequence. Primers B and C overlap by a length sufficient to permit annealing of their 5' ends to each other under conditions which allow a PCR to be performed. Thus, the amplified regions AB and CD may undergo gene splicing by overlap extension to produce the humanised product in a single reaction.

The Transfection and Expression of the Reshaped Antibody

Following the mutagenesis reactions to reshape the antibody, the mutagenised DNAs can be linked to an appropriate DNA encoding a light or heavy chain constant region, cloned into an expression vector, and transfected into host cells, preferably mammalian cells. These steps can be carried out in routine fashion. A reshaped antibody may therefore be prepared by a process comprising:

(a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a human antibody and the CDRs required for the humanised antibody of the invention;

(b) preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;

(c) transforming a cell line with the first or both prepared vectors; and

(d) culturing said transformed cell line to produce said altered antibody.

Preferably the DNA sequence in step (a) encodes both the variable domain and each constant domain of the human antibody chain. The humanised antibody can be prepared using any suitable recombinant expression system. The cell line which is transformed to produce the altered antibody may be a Chinese Hamster Ovary (CHO) cell line or an immortalised mammalian cell line, which is advantageously of lymphoid origin, such as a myeloma, hybridoma, trioma or quadroma cell line. The cell line may

also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof.

The CHO cells used for expression of the antibodies according to the invention may be dihydrofolate reductase (dhfr) deficient and so dependent on thymidine and hypoxanthine for growth (Urlaub *et al.*, Proc. Natl. Acac. Sci. U.S.A., 77 4216-4220 (1980)). The parental dhfr$^-$ CHO cell line is transfected with the DNA encloding the antibody and dhfr gene which enables selection of CHO cell transformants of dhfr positive phenotype. Selection is carried out by culturing the colonies on media devoid of thymidine and hypoxanthine, the absence of which prevents untransformed cells from growing and transformed cells from resalvaging the folate pathway and thus bypassing the selection system. These transformants usually express low levels of the DNA of interest by virtue of co-integration of transfected DNA of interest and DNA encoding dhfr. The expression levels of the DNA encoding the antibody may be increased by amplification using methotrexate (MTX). This drug is a direct inhibitor of the enzyme dhfr and allows isolation of resistant colonies which amplify their dhfr gene copy number sufficiently to survive under these conditions. Since the DNA sequences encoding dhfr and the antibody are closely linked in the original transformants, there is usually concomitant amplification, and therefore increased expression of the desired antibody.

Another preferred expression system for use with CHO or myeloma cells is the glutamine synthetase (GS) amplification system described in WO 87/04462. This system involves the transfection of a cell with DNA encoding the enzyme GS and with DNA encoding the desired antibody. Cells are then selected which grow in glutamine free medium and can thus be assumed to have integrated the DNA encoding GS. These selected clones are then subjected to inhibition of the enzyme GS using methionine sulphoximine (Msx). The cells, in order to survive, will amplify the DNA encoding GS with concomitant amplification of the DNA encoding the antibody.

Although the cell line used to produce the humanised antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used. In particular, it is envisaged that E. coli–derived bacterial strains could be used. The antibody obtained is checked for functionality. If

functionality is lost, it is necessary to return to step (2) and alter the framework of the antibody.

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be recovered and purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (See, generally, Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, a humanised antibody may then be used therapeutically or in developing and performing assay procedures, immunofluorescent stainings, and the like (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

Studies carried out by Greenwood and Clark (1993) have demonstrated that recognition of the Fc region of an antibody by human effector cells can be optimised by engineering the constant region of the immunoglobulin molecule. This could be achieved by fusing the variable region genes of the antibody, with the desired specificity, to human constant region genes encoding immunoglobulin isotypes that have demonstrated effective ADCC in human subjects, for example the IgG1 and IgG3 isotypes (Greenwood and Clark (1993) Protein Engineering of Antibody Molecules for Prophylactic and Therapeutic Applications in Man. Edited by Mike Clark, published by Academic Titles. Section II 85-113). The resulting chimeric or humanised antibodies to LMA should be particularly effective in inducing ADCC.

Antibodies with fully human variable regions against LMA can also be prepared by administering the antigen to a transgenic animal which has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled. Various subsequent manipulations can be performed to obtain either antibodies per se or analogs thereof (see, for example, US Patent No. 6,075,181).

Therapeutic methods

In one aspect the methods of the present invention utilize the antibodies or binding fragments without modification, relying on the binding of the antibodies or fragments to the surface LMAs of the myeloma cells in situ to stimulate an immune attack thereon. For example, a chimeric antibody, wherein the antigen-binding site is joined to human Fc region, e.g., IgG1, may be used to promote antibody-dependent mediated cytotoxicity or complement-mediated cytotoxicity.

In another aspect of the invention, the therapeutic method may be carried out using LMA binding moieties to which a cytotoxic agent or biological modifier is bound. Binding of the resulting conjugate to the tumor cells inhibits the growth of or kills the cells.

Anti-idiotypic monoclonal antibodies to the antibodies of the invention may also be used therapeutically in active tumor immunization and tumor therapy (see, e.g., Hellstrom *et al.*, "Anti Idiotypes" in Covalently Modified Antigens and Antibodies in Diagnosis and Therapy, supra at pp. 35-41).

In the area of multiple myeloma, the antibodies or antibody fragments of the present invention have further utility in the preparation of cellular samples from which myeloma cells have been removed. This use is particularly important in autologous bone marrow transplants, wherein a sample of bone marrow is harvested from a cancer patient prior to the patient's undergoing high-dose chemotherapy. The goal of the high dose chemotherapy is to destroy the cancer cells, which also results in the depletion of bone marrow cells. Following such treatment, the harvested bone marrow cells are reintroduced into the patient.

In myeloma and related diseases, the harvested bone marrow is contaminated with myeloma cells; thus, reintroduction of untreated bone marrow will simply reintroduce the disease. Previous methods to prevent reintroduction of cancer cells have included treatment of the bone marrow sample with chemotherapeutic agents and other anti-neoplastic agents in vitro. Other methods include purging the sample of cancer cells.

In a further practice of the present invention, the monoclonal antibodies and fragments described herein may be used to remove myeloma cells from a patient's bone marrow

sample before reintroduction into the patient. In one non-limiting example, the monoclonal antibodies, or binding fragments, are attached to a matrix, such as beads. This may be accomplished by any of several well-known methods for preparing an affinity matrix comprising antibodies or their binding fragments. The bone marrow sample is then exposed to the matrix, such as by passage of the cells over a column containing the matrix, under conditions to promote the binding of the myeloma cells in the sample through antigen/antibody interactions with the antibodies or binding fragments attached to the matrix. The myeloma cells in the sample adhere to the matrix; while the column effluent, i.e., the non-adherent cellular population, is depleted of myeloma cells. The effectiveness of the procedure may be monitored by examining the cells for residual myeloma cells, such as by using a detectably-labeled antibody as described below. The procedure may be repeated or modified to increase effectiveness.

This purging procedure (see, e.g., Ramsay *et al.*, J. Clin. Immunol., 8(2):81-88, 1988) may be performed together with other methods for removing or killing cancer cells, including, but not limited to, exposing the purified bone marrow cells to chemotherapeutic agents. Such chemotherapeutic agents include the use of the antibodies or antibody binding fragments of the present invention conjugated to a cytotoxic agent, as those described above for in vivo therapeutic treatment. Accordingly, conjugates of the antibodies or antibody fragments of the present invention with cytotoxic agents may be used for the ex vivo killing of tumor cells in a cellular sample. The methods may additionally include exposing the cells to cytokines (e.g., GM-CSF, IL-6), cytokine receptors (e.g., IL-6-receptor), mitogens (e.g., poke weed mitogen (PWM)), or adhesion molecules (e.g., CD40 ligand) in order to stimulate the myeloma cells to rapidly differentiate and thereby upregulate expression of cancer-specific antigens on their cell surface. These treatment modalities are intended to render the myeloma cells vulnerable to the *in vitro*- mediated cytotoxicity achieved by incubation with the monoclonal antibody, or fragments thereof, according to the present invention. .

In another aspect of the therapeutic methods of the present invention, the antibodies, or binding fragments thereof, conjugated with cytotoxic agents, such as chemotherapeutic agents, a photo-activatable toxin, or a radionuclide, may be used *in vitro* or *ex vivo* to inhibit or kill myeloma cells from a bone marrow sample, in the absence of the purging technique described above. The treatment of a sample with the cytotoxic antibodies, or antibody fragments, may be combined with other methods to kill cancer cells to

increase the effectiveness of a bone marrow transplant, particularly an autologous bone marrow transplant, by removing cells from the tissue to be transplanted. These methods may include additionally exposing the cells to cytokines, etc. Thus, a method is described herein for removing myeloma cells from a isolated cellular sample comprising the steps of exposing the cellular sample to a solid matrix on which a monoclonal antibody, or antibody binding fragment as described herein, is bound under conditions in which the myeloma cells adhere to the monoclonal antibody, or binding fragment thereof, and isolating a cellular fraction of the cellular sample which does not bind to the matrix. By way of non-limiting example, bone marrow cells are used, particularly for a transplant, and preferably, an autologous bone marrow transplant.

As will be appreciated by those skilled in the art, some myeloma patients have significant levels of free lambda light chain in their circulation. As anti-LMA antibodies react with these free light chains, their presence in the fluid of the subject may reduce the efficiency of the treatment. Accordingly, in a preferred embodiment of the invention the method of treatment further comprises the step of treating the subject to reduce the levels of free lambda light chains circulating in the fluid (e.g. blood) of the subject prior to administration of the anti-LMA antibody. This additional treatment step may involve, for example, plasmapheresis. As will be known by those skilled in the art, plasmapheresis is a process in which the plasma is removed from blood cells by a device known as a cell separator. The separator works either by spinning the blood at high speed to separate the cells from the fluid or by passing the blood through a membrane with pores so small that only the plasma can pass through. The cells are returned to the subject, while the plasma, which contains the free kappa light chains, is discarded and replaced with other fluids. Medication to keep the blood from clotting (e.g. an anticoagulant) may be given through a vein during the procedure.

It will be appreciated that methods of treating lymphoproliferative disorders such as multiple myeloma involving the use of anti-LMA antibodies may be performed in isolation or as an adjunct to known chemotherapy or radiotherapy regimes. For example, anti-LMA antibody treatment may be conducted in conjunction with or after treatment with drugs such as melphalan or cyclophosphamide.

Pharmaceutical Compositions, Dosages, and Routes of Administration

The present invention is also directed to pharmaceutical compositions comprising an anti-LMA antibody together with a pharmaceutically-acceptable carrier or diluent.

The antibodies and pharmaceutical compositions of the invention are useful for parenteral, topical, oral, or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment. The preferred route of administration of anti-LMA antibodies is parenteral; as used herein, the term "parenteral" includes intravenous, intramuscular, subcutaneous, rectal, vaginal or intraperitoneal administration. Of these, intravenous administration is most preferred.

The compositions for administration will commonly comprise a solution of the antibody dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of antibody in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs.

The growth of tumour cells may be inhibited or reduced by administering to a subject in need of the treatment an effective amount of anti-LMA antibody. Typically, the antibody may be administered in an amount of about 0.001 to 2000 mg/kg body weight per dose, and more preferably about 0.01 to 500 mg/kg body weight per dose. Repeated doses may be administered as prescribed by the treating physician. However, other amounts are also suitable. Generally, the administration of the antibody is conducted by infusion so that the amount of antibody present that may produce a detrimental effect may be kept under control by varying the rate of administration. Typically, the infusion of one dose may last a few hours. However, also contemplated herein is the constant infusion of a dose for therapeutic purposes that will permit the maintenance of a constant level of the antibody in serum. The infusion of the anti-

LMA antibody may be conducted as follows. Intravenous (I.V.) tubing may be pretreated, e.g., with 0.9% NaCl and 5% human serum albumin and placed for intravenous administration. The I.V. infusion may comprise a total volume of 250 ml of 0.9% NaCl and 5% human serum albumin and be infused over a period of about 2 hours depending on any rate-dependent side effects observed. Vital signs should be taken, for example, every fifteen minutes during the infusion and every one hour post infusion until stable. A thorough cardiopulmonary physical examination may be done prior to, and at the conclusion, of the infusion. Medications including acetaminophen, diphenhydramine, epinephrine, and corticosteroids may be kept at hand for treatment of allergic reactions should they occur. The administration of the antibody may be repeated as seen desirable by a practitioner.

In any treatment regimen, the therapeutic composition may be administered to a patient either singly or in a cocktail containing other therapeutic agents, compositions, or the like, including, but not limited to, immunosuppressive agents, tolerance-inducing agents, potentiators and side-effect relieving agents. Particularly preferred are immunosuppressive agents useful in suppressing allergic reactions of a host. Preferred immunosuppressive agents include prednisone, melphalain, prednisolone, DECADRON (Merck, Sharp & Dohme, West Point, Pa.), cyclophosphamide, cyclosporine, 6-mercaptopurine, methotrexate, azathioprine and i.v. gamma globulin or their combination. Preferred potentiators include monensin, ammonium chloride, perhexiline, verapamil, amantadine and chloroquine. All of these agents are administered in generally accepted efficacious dose ranges such as those disclosed in the Physician's Desk Reference, 41st Ed., Publisher Edward R. Barnhart, N.J. (1987). Patent Cooperation Treaty (PCT) patent application WO 89/069767 published on Aug. 10, 1989, which is incorporated by reference herein.

Diagnostic Assays and Kits

The antibodies of the present invention are also useful for diagnostic applications, both *in vitro* and *in vivo*, for the detection of human multiple myeloma cells. *In vitro* diagnostic methods include immunohistological detection of tumor cells. Immunohistochemical techniques involve staining a biological specimen such as tissue specimen with the antibody of the invention and then detecting the presence of antibody complexed to its antigen as an antigen-antibody complex. The formation of such antibody-antigen complexes with the specimen indicates the presence of multiple

myeloma cells in the tissue. Detection of the antibody on the specimen can be accomplished using techniques known in the art such as immunoenzymatic techniques, e.g., immunoperoxidase staining technique, or the avidin-biotin technique, or immunofluorescence techniques (see, e.g., Ciocca *et al.*, "Immunohistochemical Techniques Using Monoclonal Antibodies", Methods Enzymol, 121:562-79, 1986 and Kimball, (ed), Introduction to Immunology (2.sub.nd Ed), pp. 113-117 (Macmillan Pub. Co., 1986).

In a preferred embodiment, detection is by the detection of a label bound to the fusion polypeptide. Means of labeling polypeptides are well known to those of skill in the art. Labels may be directly linked through a covalent bond or covalently through a linking molecule which typically bears reactive sites capable of forming covalent bonds with the label and the antibody respectively. a common approach is to label the polypeptide and the label with either avidin or streptavidin or biotin which, in turn, bind irreversibly with each other.

Suitable labels are well known to those of skill in the art. The term "label", as used herein, refers to a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive molecules such as ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{35}S, fluorescent dyes such as fluorescein or rhodamine, electron-dense reagents, isothiocyanate; chromophores, enzymes (as commonly used in an ELISA), luminescent enzymes such as luciferase and the like.

Such labeled antibodies or binding fragments may be used for the histological localization of the antigens, for ELISA, for cell sorting, and for other immunological techniques to detect and/or quantify the antigens, and cells bearing the antigens, for example. As noted above, a particular use of such labeled antibodies, or fragments thereof, is in determining the effectiveness of myeloma cell depletion from bone marrow tissue prior to transplant, particularly autologous bone marrow transplant.

The present invention is also directed to imaging methods for multiple myeloma using anti-LMA antibodies as described hereinabove. Other cancers bearing the LMA are also amenable to these diagnostic procedures. The method involves administration or infusion of monoclonal antibodies or binding fragments as described herein, with or without conjugation to a detectable moiety, such as a radionuclide. After

administration or infusion, the antibody, or antibody fragment, binds to the tumor cells, after which the location of the antibodies, or fragments, is detected. For detectably-labeled antibodies or their binding fragments, such as those labeled with a radionuclide, imaging instrumentation may be used to identify the location of the agent within the body. For use of unlabeled antibodies or fragments, a second, detectable reagent may be administered which locates the antibodies or antibody fragments, and thus may be suitably detected. These methods have been used for other antibodies, and the skilled artisan will be amply aware of these various methods for imaging the location of antibodies or fragments within the body.

Detection of the anatomic location of LMA bearing cancer cells can be useful for the subsequent planning of antitumor therapy in each particular patient. In particular, immunohistochemical pathologic diagnosis in tissue sections (e.g., biopsies), fluid samples (e.g., blood) or cytological preparations can be performed using the fusion polypeptides of the present invention.

This invention also embraces kits for research or diagnostic purposes. A kit typically includes one or more containers containing an anti-LMA antibody. The anti-LMA antibody may be derivatized with a label or, alternatively, it may be bound with a secondary label to provide subsequent detection. As described above, such labels may include radiolabels, fluorescent labels, enzymatic labels, i.e., horseradish peroxidase (HRP), or the like. The kit may also contain appropriate secondary labels (e.g., a sheep antimouse-HRP, or the like). The kit may also contain various reagents to facilitate the binding of the fusion polypeptides, the removal of non-specific binding antibodies, and the detection of the bound labels. Such reagents are well known to those of skill in the art.

In a further aspect of the present invention, compositions are provided which comprise the monoclonal antibody, or antibody binding fragment as described herein, bound to a solid support. A solid support for use in the present invention will be inert to the reaction conditions for binding. A solid phase support for use in the present invention must have reactive groups or activated groups in order to attach the monoclonal antibody or its binding partner thereto. In another embodiment, the solid phase support may be a useful chromatographic support, such as the carbohydrate polymers SEPHAROSE.RTM., SEPHADEX.RTM., or agarose. As used herein, a solid phase support is not limited to a specific type of support. Rather, a large number of supports

are available and are known to one of ordinary skill in the art. Solid phase supports include, for example, silica gels, resins, derivatized plastic films, glass beads, cotton, plastic beads, alumina gels, magnetic beads, membranes (including, but not limited to, nitrocellulose, cellulose, nylon, and glass wool), plastic and glass dishes or wells, etc.

Methods for using the research and diagnostic kits described above are generally well known, and are generally provided in an instruction manual for use of the kit.

In order that the present invention may be more clearly understood preferred forms will be described with reference to the following non-limiting examples.

Experimental Details

Antibody

The murine monoclonal antibody (mAb) against human lambda light chains was obtained from Research Diagnostics, Inc (RDI; Flanders, NJ, USA). This mAb is RDI-TRK1L7-3D12, and is designated herein as L7.

Cell line

A human lambda-type multiple myeloma cell line (LP-1) was obtained from the DSMZ cell bank facility in Germany. Cells were maintained in Iscoves MDM and 20% FBS at 37 °C with 5% CO_2.

ELISA

The specificity of L7 for human free lambda light chains was confirmed using an ELISA. Antigens consisted of Bence-Jones proteins that had been isolated from the urine of patients with multiple myeloma. Specifically, to different human free lambda light chains that had been purified as monomer and dimer fractions (Lam F and Lam H) and a monomer and dimer mixture of human free lambda light chains (MOS). Lambda light chains associated with heavy chain were represented by intact human gamma globulins (Sigma). An irrelevant control antigen consisted of human free kappa light chains (VOR) in both monomer and dimer form.

The wells of an ELISA plate were coated with each antigen in phosphate buffered saline pH 7.4 with 0.02% sodium azide (PBS-az). After incubation at 37 °C for 1 hour, the wells were washed three times with PBS-az and blocked with 3% BSA in PBS-az overnight at 4 °C. The antibody, L7, was then added to the wells. Following incubation at 37 °C for 3 hours the wells were washed three times with PBS-az. A goat anti-mouse IgG-AP conjugate (Sigma) was added to each well and incubated for 1 hour at 37 °C. Finally the wells were washed as described above and the substrate (BCIP/NBT; Sigma) was added to each well. The colour development was measured at 405 nm on an ELISA plate reader.

Flow Cytometry

Binding of L7 to LP-1 myeloma cells was evaluated by flow cytometry. LP-1 cells were harvested, washed by centrifugation and resuspended at a density of 1 x 10^6 cells/mL in PBS with 1% BSA-az. Aliquots of 5 x 10^5 cells were pelleted and then incubated with L7 (100 µg/mL) for 30 minutes on ice. The control samples consisted of L7 (100 µg/mL) that had been pre-incubated with 400 µg/mL of free lambda light chains (Lam F) or free kappa light chains (VOR) for 1 hour at 37 °C. After incubation with the antibody, cells were washed twice in 750 µL of PBS with 1% BSA-az and incubated in 50 µL of a 1:20 dilution of PE conjugated goat anti-mouse $F(ab')_2$ (Dako) for 30 minutes on ice. Cells were washed twice before analysis by flow cytometry on a FacScan (BD Biosciences).

Lambda light chain inhibition of L7 Binding to LP-1 cells

L7 (100 µg/mL) was pre-incubated with increasing concentrations of free lambda light chain dimers (Lam H) or with VOR (800 µg/mL) as described above. After incubation the antibody mixtures were added to 5 x 10^5 cells and binding was determined by flow cytometry as described above.

Results

The specificity of L7 binding to human free lambda light chains is shown in Figure 1. These results indicate that the antibody binds to both the monomer and dimer forms of two different lambda light chains, Lam F and Lam H. The antibody does not bind to

lambda light chains associated with heavy chain in the human gamma globulin nor does it bind free kappa light chains. L7 does not bind to the lambda light chain designated MOS. This may be related to a structural feature of MOS that prevents or blocks the binding of the antibody.

Flow cytometry results indicate that L7 binds to a cell surface molecule on LP-1 cells (Figure 2). Antibody binding to the cells was inhibited by monomers of free lambda light chain (Lam F) but was not inhibited by an equivalent concentration of free kappa light chain. Results shown in Figure 3 indicate that pre-incubation of L7 with a range of Lam H concentrations (200-800 µg/mL) specifically inhibited binding of the antibody to LP-1 cells. The binding of L7 to LP-1 cells was inhibited in the presence of 800 µg/mL of Lam H.

Conclusion

The murine monoclonal antibody, L7, binds specifically to two different human free lambda light chains and does not bind to lambda light chains associated with heavy chain. Analysis of antibody binding to the lambda type myeloma cell line, LP-1, indicates that the antibody binds to a cell surface antigen. Binding to the cell surface antigen can be blocked by pre-incubating the antibody with two different lambda light chains. In addition, the soluble form of free lambda light chain can completely abrogate binding of L7 to the cell surface antigen on LP-1 cells. These data suggest that the antibody recognises a cell surface antigen that contains similar epitopes found on free lambda light chains.

The experiments detailed herein demonstrate that the murine monoclonal antibody against free lambda light chains, designated L7 binds to an antigen on the cell surface of a lambda myeloma cell line, LP-1. We propose that this LMA consists of free lambda light chains associated with the cell membrane and can be used to specifically target myeloma cells from patients with lambda type multiple myeloma using monoclonal antibodies.

As LMA is unique to the cell membrane of the malignant B-cell it is proposed that any mAb that is capable of selectively binding these antigens will be useful in the treatment of diseases such as multiple myeloma. As a secondary effect any mAb of the appropriate isotype that is capable of binding LMA will be able to induce cell death by

using the host effector cells to bring about ADCC. This secondary effect will aid the depletion of malignant cells in patients with lymphoproliferative disorders.

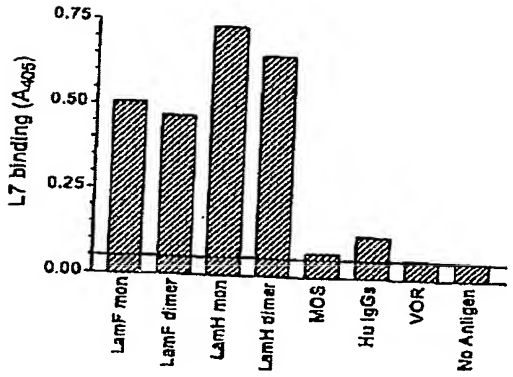
All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are apparent to those skilled in molecular biology or related fields are intended to be within the scope of the invention.

Dated this twenty sixth day of February 2004

PacMab Pty Ltd

Patent Attorneys for the Applicant:

F B RICE & CO


0.75
0.50
0.25
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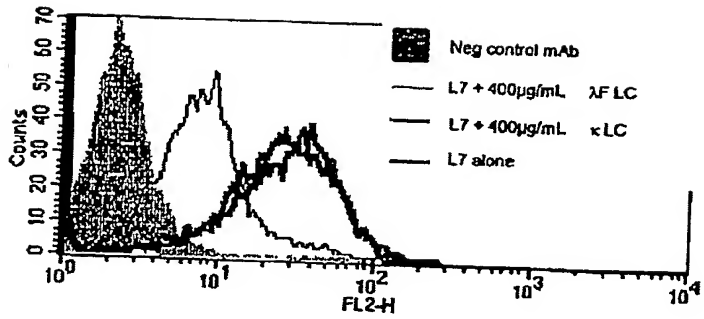
LamF dimer
LamH mon
LamH dimer
MOS
Hu IgGs
VOR
No Antigen

Figure 1


Counts
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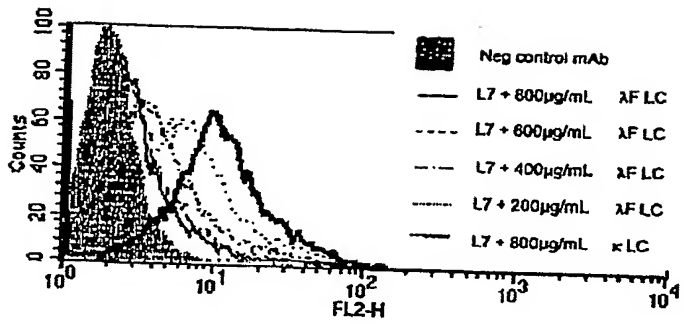
10^0 10^1 10^2 10^3 10^4
FL2-H
Neg control mAb
L7 + 400µg/mL λF LC
L7 + 400µg/mL κLC
L7 alone

Figure 2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20
40
60
80
100
10^0
10^1
10^2
10^3
10^4
FL2-H
Neg control mAb
L7 + 800µg/mL λF LC
L7 + 600µg/mL λF LC
L7 + 400µg/mL λF LC
L7 + 200µg/mL λF LC
L7 + 800µg/mL κ LC

Figure 3